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Minireview

Oviductal structure and provision of egg envelops in the apple snail *Pomacea canaliculata* (Gastropoda, Prosobranchia, Ampullariidae)

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The apple snail *Pomacea canaliculata* (Lamarck, 1822) is an amphibious oviparous dioecious freshwater gastropod displaying internal fertilization. During the breeding season, just after copulation, the females lay pink-reddish calcareous-shelled egg clusters above water level, on the emerging parts of plants, rocks and other elements (Albretch *et al.*, 1996; Andrews, 1964; Estebenet and Cazzaniga, 1993; Hylton Scott, 1957).

In *Pomacea canaliculata* the female reproductive system exhibits an organization similar to that of other prosobranch gastropods (Andrews, 1964; Buckland-Nicks and Chia, 1990; Carvalho Thiengo, 1987, 1989, 1993; Hylton Scott, 1957; Hyman, 1967; Keawjam, 1987; Schulte-Oehlmann *et al.*, 1994), consisting of a single, tree-branched ovary and a rather complex oviduct. The ovary is located superficially on the collumelar axis of the visceral hump closely attached to the digestive gland. Its whitish tubules are lined by a germinal epithelium composed of oogenic and follicular cells. The oogenic cell line comprises oogonia and previtellogenic and vitellogenic oocytes (Bedford, 1966; Cruz López *et al.*, 2000; Hodgson and Eckelbarger, 2000; Martin, 1986). The main ovarian tubules fuse to form the ovarian segment of the oviduct that runs to the base of the spire. The latter, according to its ontogenetic origin, can be divided into three sections: the ovarian, renal and pallial parts. A short renal segment follows the ovarian oviduct, the lumen of both being lined by a ciliated epithelium. In the renal section the cells are taller and the cilia longer than in the ovarian segment, its lumen being almost obliterated by the folding of its walls. These histological differences demarcate precisely that part of the duct, which is renal in origin. This identification being confirmed by its position adjacent to the visceral ganglion, as well as by the presence of a small diverticulum directed towards the pericardial cavity, apparently a vestige of the gonopericardial duct (Andrews, 1964). In contrast with the ovarian and renal segments, the last section of the genital tract, the pallial oviduct, exhibits a high degree of development and structural complexity probably related to its par-

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ticipation in the complex reproductive processes, specifically in the transport and storage of sperms, fertilization, provision of egg envelopes and spawning.

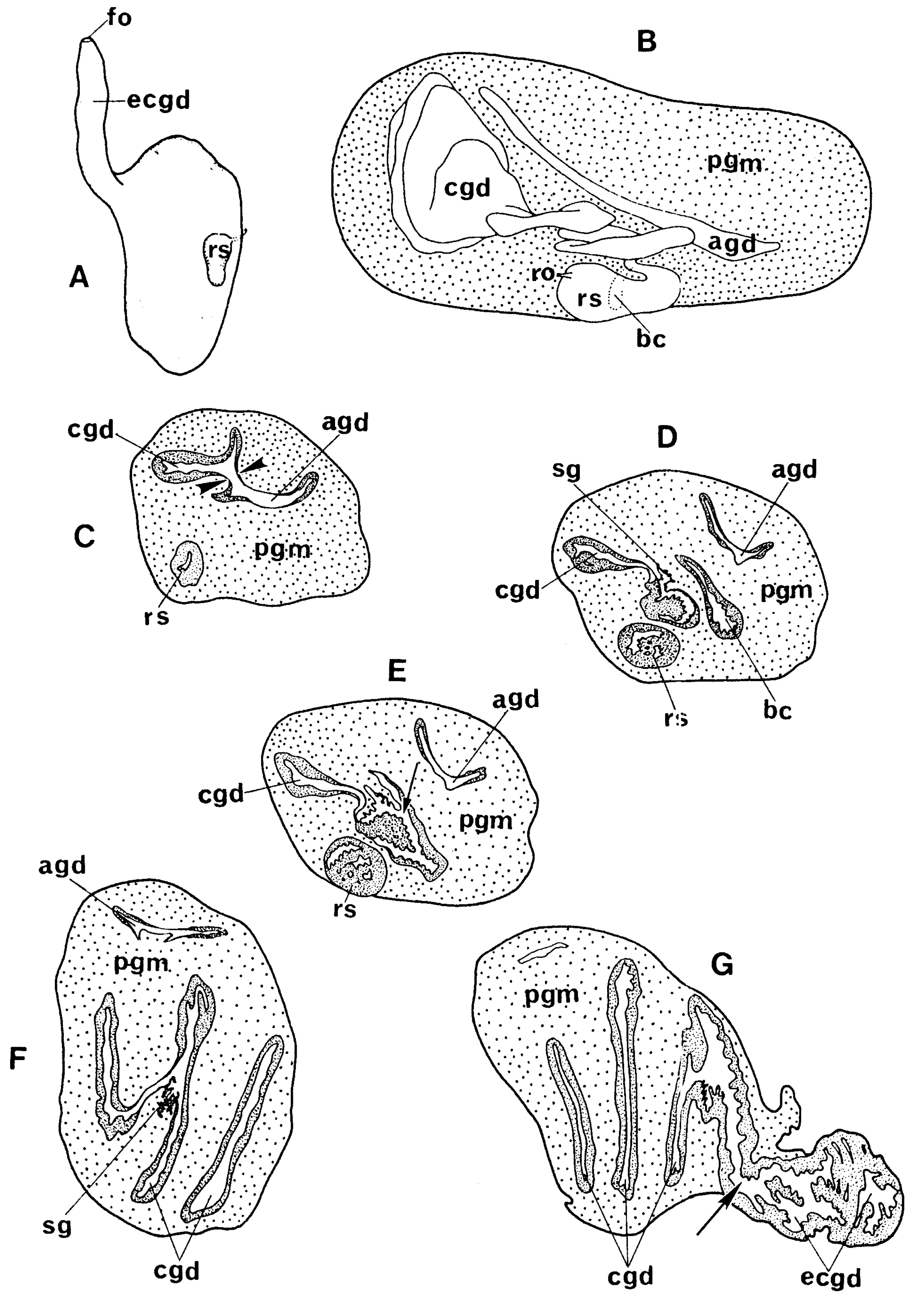
There has been a great diversity in the literature over the naming of the different segments of the pallial oviduct of prosobranch molluscs (Andrews, 1964; Buckland-Nicks and Chia, 1990; Carvalho Thiengo, 1987, 1989, 1993; Hylton Scott, 1957; Hyman, 1967; Keawjam, 1987; Schulte-Oehlmann *et al*., 1994). In general, we have followed the terminology used by Andrews (1964) and Meenakshi *et al*. (1974). In that way, in *Pomacea canaliculata*, the pallial oviduct is constituted by a receptaculum seminis, a bursa copulatrix and an albumen gland-capsule gland complex (Catalán and Moreno, 1984; Catalán *et al*., 1996, 2000; Winik *et al*., 1998), which merge in a large, pink-reddish pear-shaped structure called uterus by Hylton Scott (1957). The posterior two thirds of this structure are embedded in the visceral mass while the anterior third protrudes in the posterior wall of the mantle cavity. The distal portion of the capsule gland duct emerges from the right part of the uterus forming an arched tubular structure that continues along the mantle skirt and opens anteriorly near the anus. This last extrauterine segment of the pallial oviduct is the vagina described by Hylton Scott (1957) (Fig. 1).

The complex spatial distribution of the different segments of the pallial oviduct of *Pomacea canaliculata* does not appear in other ampullarids such as *Pila virens*, *P. ampullacea*, *P. angelica*, *P. gracilis*, *P. pesmei*, *P. polita* and *Marisa cornuarietis*. In these species the distribution is more simple since the different segments are topographically arranged in a sequential manner, that is, one after the other (Keawjam, 1987; Schulte-Oehlmann *et al*., 1994).

The receptaculum seminis is constituted by a bulbous structure and a U-curved duct which are partially embedded in the ventral wall of the albumen gland-capsule gland complex. It is composed of differently organized regions and enclosed in a thick coat of connective and muscular fibers. The proximal bulbous region has an internal coiled section as a continuation of the renal oviduct. The medial bulbous region exhibits a glandular and a non-glandular area. The former is characterized by a large number of branched pouches lined by a simple epithelium with both ciliated and large glandular cells. The use of specific staining for the detection of mucopolysaccharides and mucoproteins (PA-Schiff; Alcian blue pH 2.5 and pH 0.5; Hale - PA-Schiff; Toluidine blue pH 5.6) demonstrated the heterogeneity of the glandular contents. The sacs in the non-glandular region store sperm with heads arranged perpendicular to the surface of the epithelium. The distal bulbous region has pouches with an irregular lumen that will continue with the U-shaped duct of the receptaculum seminis. Finally, the lumen of this duct contacts with the lumen of the bursa copulatrix and the capsule gland duct (Moreno and Catalán, 1985). According to Andrews (1964) the function of the coiled oviduct is to separate groups of oocytes coming from the ovary into a single line in order to ensure fertilization.

Since sperm can be stored for long periods in the receptaculum seminis, even from one reproductive season to the next, it may be assumed that the products of the glandular area would serve as nutrients for sperm survival. Estebenet and Cazzaniga (1993) and Albretch *et al*. (1999) have reported that females may spawn fertile eggs, even after prolonged periods after mating (up to from one reproductive season to the other). Our observations lead us to think that the glandular region might release substances that participate in the capacitation and activation of fertilizing sperm. The positive reaction of the epithelium to alkaline phosphatase and the orderly arrangement of the sperm suggest that old and/or infertile sperm would disintegrate in the non-glandular area (Moreno and Catalán, 1985). Similar

FIGURE 1. Schematic drawings of the pallial oviduct.
A. External morphology of the albumen gland-capsule gland complex. Ventral view. **B.** Model of the complex spatial distribution of the different intraparenchymal structures of the pallial oviduct. **C- G.** Transversal sections showing the disposition of the albumen gland duct and capsule gland duct in relation to bursa copulatrix and receptaculum seminis. Drawings were obtained from stained paraffin sections. C: Arrow-heads indicate the connection between the capsule gland duct and the albumen gland duct. E: The section illustrates the joint of the receptaculum seminis duct with the bursa copulatrix (arrow). G: Arrow indicates the connection between the intraparenchymal and extraparenchymal segments of the capsule gland duct.
agd: albumen gland duct; bc: bursa copulatrix; cgd: intraparenchymal capsule gland duct; ecgd: extraparenchymal capsule gland duct; fo: female opening; pgm: parenchymal glandular mass; ro: renal oviduct; rs: receptaculum seminis; sg: sperm groove.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results were reported by Schulte-Oehlmann *et al.* (1994) for *Marisa cornuarietis*.

The thick muscular-connective coat of the receptaculum seminis would control not only the flow of sperm into the receptaculum but also discharge of the fertilized oocytes towards the lumen of the albumen gland-capsule gland complex. The ciliar movement of the epithelial cells would also contribute to the transit of gametes and secretions.

The bursa copulatrix communicates directly with the receptaculum seminis and with the proximal end of the sperm groove of the capsule gland duct, which transports the seminal fluid during mating. It is lined by a columnar ciliated epithelium with numerous folds and surrounded by circular muscle fibers. Summarily, the bursa copulatrix is a pouch into which the sperm groove discharges sperms and seminal fluids temporarily stored inside the bursa, while the seminal receptacle, capable of facilitating sperm survival for long periods of time, is the site where oocytes fertilization occurs.

The albumen gland-capsule gland complex is involved in the formation of the envelopes surrounding the fertilized oocytes. The parenchymal glandular mass exhibits tubular-acinous adenomeres formed by two cell types: albumen secretory cells and labyrinthic cells alternately arranged and joined by zonulae adherens and septate junctions (Fig. 2). Smooth muscle fibers and neurosecretory nerve terminals are found in the interstice in close proximity to the adenomeres.

The albumen secretory cells, voluminous and ovoidal or pyramidal in shape, present numerous short microvilli in their apical surfaces. These cells are characterized by electron-lucid basal nuclei with prominent nucleoli, a well-developed rough endoplasmic reticulum, conspicuous Golgi complexes and abundant secretory granules (Figs. 3, 4). Histochemically, the albumen synthesized by these cells would be a non-sulphated acidic periodate reactive glycoprotein. Another distinctive morphological feature is the presence of an electron-dense material that might be deposits of intracellular calcium that gradually infiltrates the cytoplasmic matrix, the cellular organelles and the secretory granules, originating large calcareous spherules with a concentric laminar structure (Catalán *et al.*, 1996; Winik *et al.*, 1998) (Fig. 5). Results obtained in *Pomacea paludosa* by Meenakshi *et al.* (1974) under the scanning electron microscope demonstrated that these spherules are also composed of several concentric layers formed by fusion of small crystallites.

The labyrinthic cells, located between the albumen secretory ones, exhibit deep folds of the basal plasma membrane that extend through the cellular cytoplasm up to the subapical region, making up a remarkable basal labyrinth. The complex extracellular space limited by the basal labyrinth shows electron-dense calcium material. Mitochondria are the most numerous and conspicuous organelles lodged in the narrow cytoplasmic compartments of these cells. The nuclei, oval in shape, are basally located and exhibit clumps of heterochromatin. On the luminal surfaces cilia intermingled with few short microvilli are visible (Catalán *et al.*, 1996; Winik *et al.*, 1998) (Fig. 6).

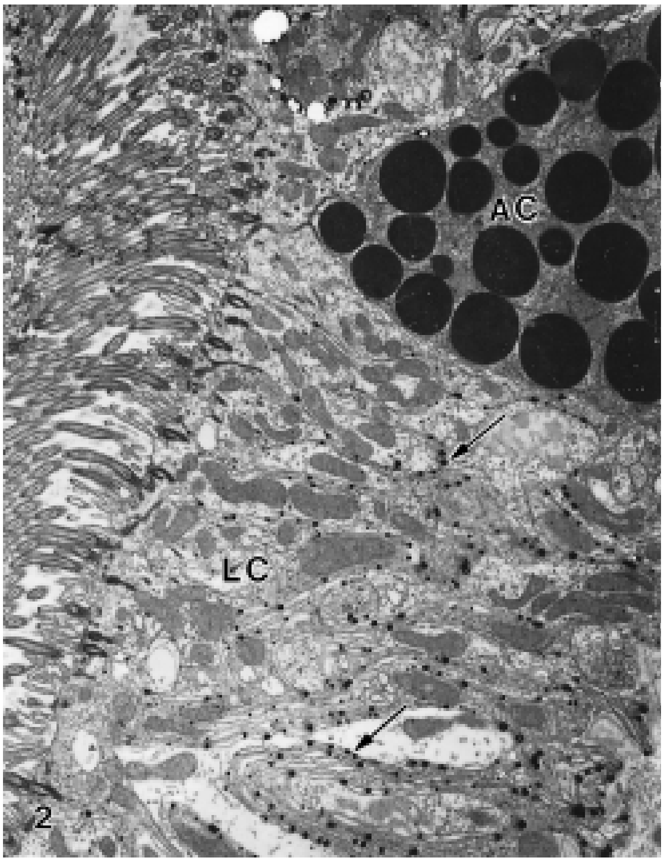
The materials produced by the parenchymal cells and the calcium circulating through the extracellular space of the labyrinthic cells pass through tributary ducts towards the main albumen gland duct. The latter is indeed a flattened sac that extends along the dorsal region of the parenchymal glandular mass. The albumen gland duct is lined by an epithelial layer composed of three cell types: ciliated, secretory and neurosecretory-like cells, which rest on a basal membrane. The epithelial layer becomes thicker at the lateral edges of the duct

FIGURE 2. Acinus of the parenchymal glandular mass. Albumen secretory (AC) and labyrinthic cells (LC) outlining the ductal lumen. Numerous elongated mitochondria are present in the cytoplasmic folds of the labyrinthic cells. Electron-dense calcium deposits can be seen in the extracellular spaces (arrows). (X 5,600)

FIGURE 3. Acinus of the parenchymal glandular mass. Note the pyramidal shape of the albumen secretory cell. Secretory granules of different sizes and shapes fill the whole cytoplasm. The nucleus (N), peripherally and basally located, exhibits a prominent nucleoli. (X 7,800)

FIGURE 4. Albumen secretory cell. The rough endoplasmic reticulum is arranged in a concentrical pattern. Note the electron-dense deposits (arrows) that infiltrate the cytoplasmic matrix and the cellular organelles. Secretory granules (S). (X 14,200)

FIGURE 5. Note a conspicuous calcareous spherule (arrows). Albumen secretory cells (AC). (X 5,600)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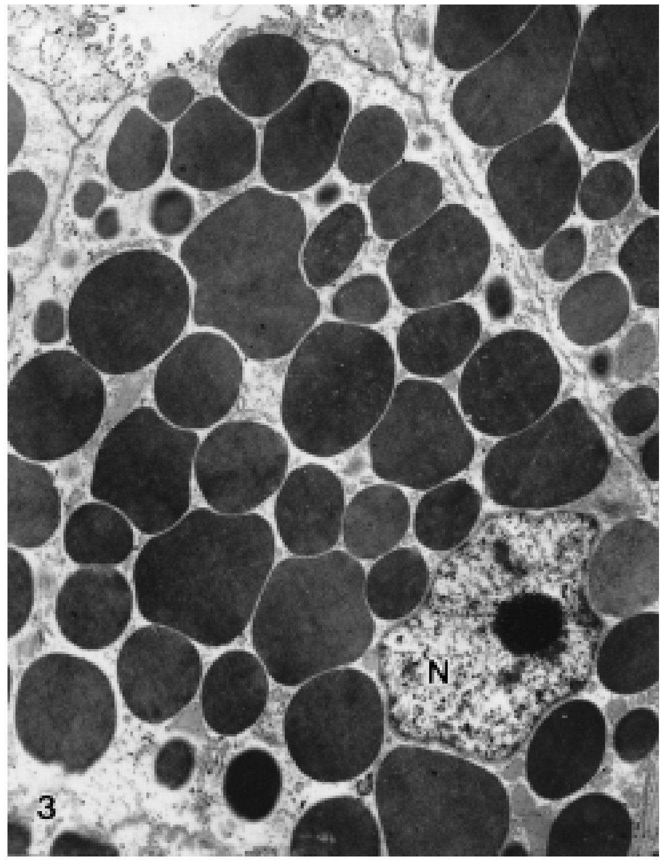
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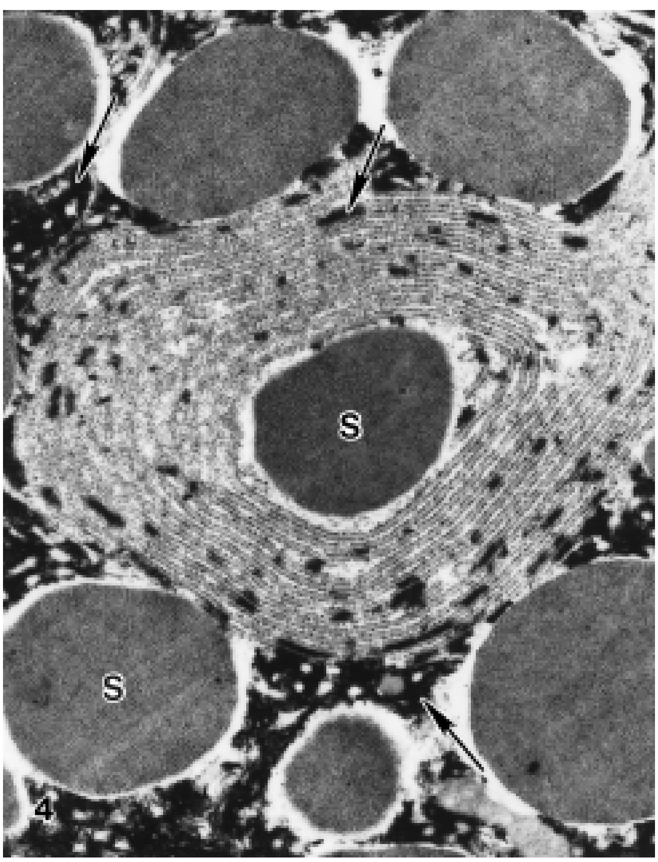
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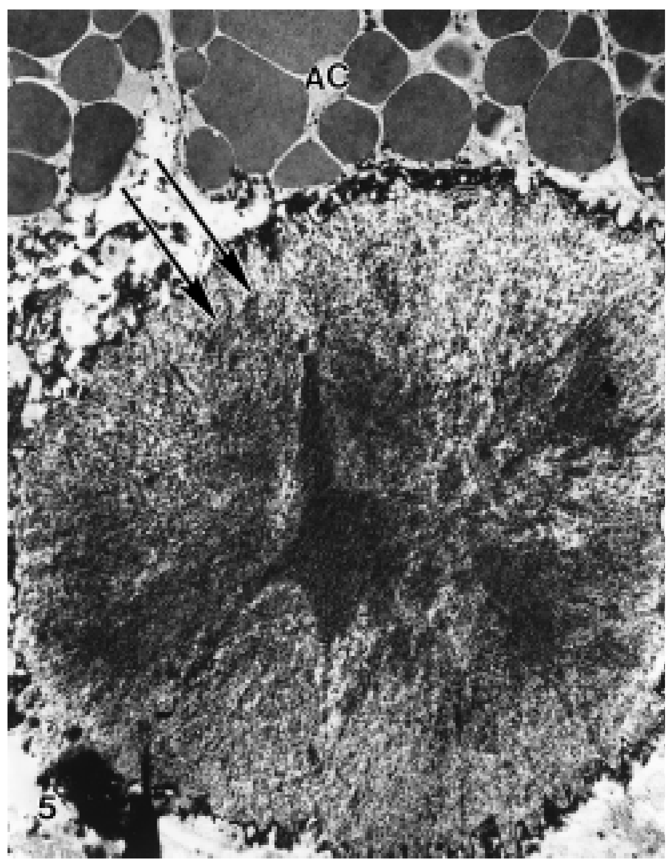
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(Fig. 7) and thinner at its dorsal and ventral surfaces due principally to variations in the volume and number of the secretory cells (Fig. 8). These cells are bottle-shaped unicellular glands with short apical microvilli, a voluminous body embedded in the parenchymal mass and a wide neck located between the ciliated cells (Fig. 7). Their nuclei are electron-lucid and basally located. The rough endoplasmic reticulum and the Golgi complex are involved in the formation of the secretory granules whose size, shape and electron density varies according to the histochemical nature of their contents (Winik *et al*., 1998). Although secretory cells that produce mucoproteins and strongly acidic non-periodate reactive mucopolysaccharides (sulphomucines) are predominant, a smaller proportion of cells that produce acidic periodate reactive mucines and only a few that secrete neutral mucines can be also observed.

The columnar ciliated cells, which show a typical ultrastructural organization with basal nuclei, numerous mitochondria and long apical cilia intermingled with short microvilli, are joined to the secretory cells by adherens and septate junctions.

The neurosecretory-like cells, basally located, present an irregular shape with lobed nuclei, and few organelles, except for numerous small membrane-bound dense-cored vesicles.

Muscle fibers, collagen fibers and nerve terminals are found in close proximity with the ductal epithelium.

The histochemical and ultrastructural characteristics of the albumen gland duct of *Pomacea canaliculata* that were described above are similar to those reported by Hinsch and Vermeire (1990) for *Pomacea paludosa*.

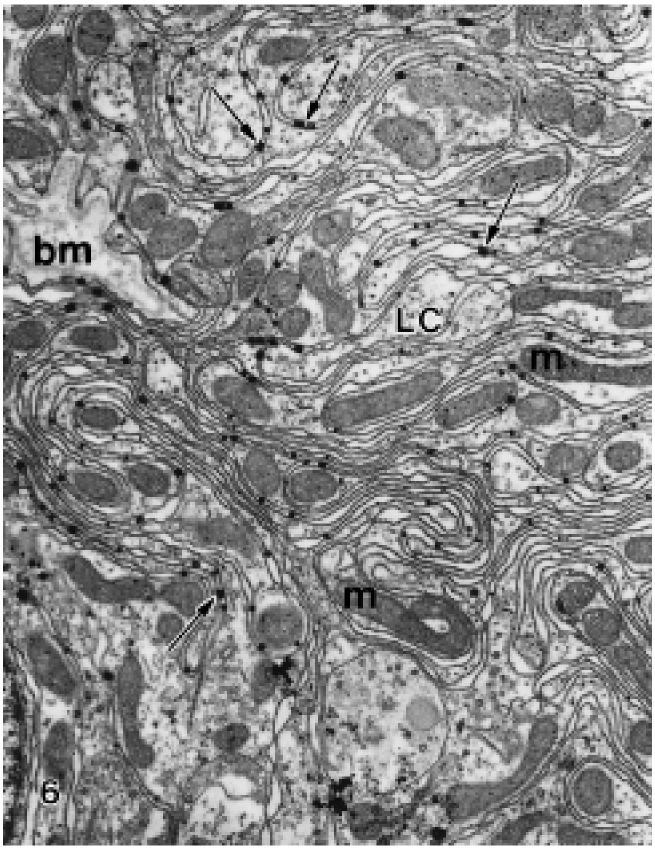
The products released by the parenchymal cells and by the secretory cells of the albumen gland duct drain towards the capsule gland duct. The latter presents along the whole course of it, from the region of the receptaculum seminis to the genital aperture, a sperm groove separated from the egg channel by a longitudinal fold. The proximal region of the coiled capsule gland duct is embedded in the parenchymal mass forming six flattened sacs showing distinct morphological and histochemical characteristics. At the level of the wide egg channel, the epithelial layer lining the ductal lumen is composed by two cell types: ciliated and secretory cells, joined by adherens and septate junctions. Columnar ciliated cells show a typical morphology which may be correlated to their kinetic function. They are either cylindrical or wedge-shaped, with basal nuclei and numerous mitochondria concentrated at the cell apex in association with the basal bodies and rootlets of the long apical cilia. The large bottle-shaped secretory cells are unicellular glands with apical microvilli, a voluminous elongated body embedded in the stroma and a wide neck located between the ciliated cells. They exhibit a basal electron-lucid nucleus and numerous secretory granules that fill up the cytoplasm. In the first four sacs the epithelial secretory cells of the two opposing halves of the duct synthesized histochemically different products, a fact probably related with the morphological variability observed in the secretory granules at the ultrastructural level (Figs. 9, 10). The secretory cells of one of the halves produce principally acidic mucopolysaccharides and some mucoproteins as well, while those of the other secrete a protein product as well as some mucopolysaccharides (Catalán *et al*., 2001). Studies tending to correlate these observations with the ultrastructural organization are in progress.

In the last two sacs of the coiled capsule gland duct two types of secretory cells alternately arranged throughout the epithelial layer can be observed. One of them produce acidic mucopolysaccharides (sulphomucines and phosphomucines) while the other presents a basal inclusion that would be a complex scleroprotein. Calcareous deposits fill the cytoplasm of certain glandular ephitelial cells as well as the ductal lumen (Figs. 11, 12).

FIGURE 6. Labyrinthic cells (LC). Note the basal labyrinth folds with profuse mitochondria (m). Spherules of electron-dense calcium deposits (arrows) can be seen in the complex extracellular spaces. Basal membrane (bm). (X 12,300)

FIGURE 7. Albumen gland duct. Panoramic view of the epithelial layer at the lateral edge of the duct. Secretory cells (SC) are interspersed between ciliated cells (CC). Arrows indicate the cytoplasmic bridges that join the cellular necks to the main bodies of the secretory cells. (X 5,700)

FIGURE 8. Albumen gland duct. Note the thinning of the epithelial layer at the dorsal surface of the duct. Secretory cell (SC). Ciliated cells (CC). Arrows indicate prominent lysosomal structures. (X 12,800)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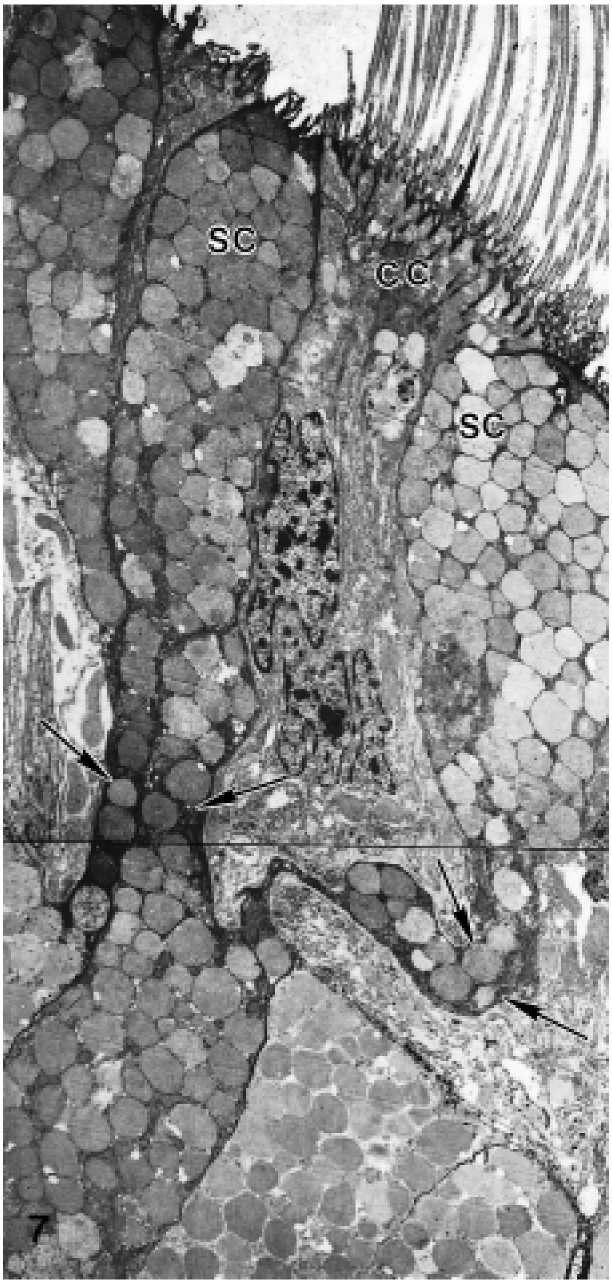
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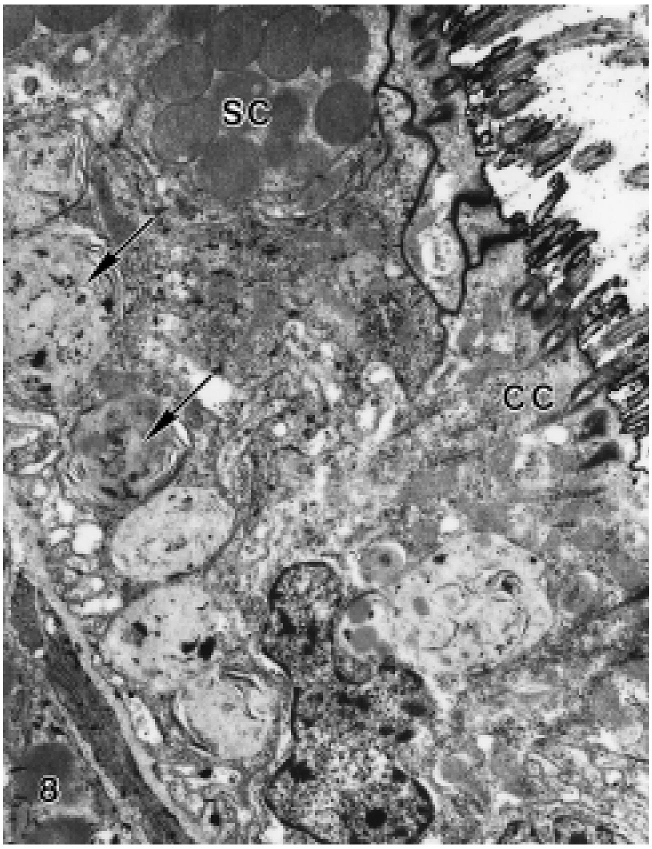
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The sperm channel is a functional duct with a narrow lumen outlined by folded epithelial crests, surrounded by a few muscle and connective fibers. During mating, the seminal fluid in transit widens the sperm channel that transports the sperm directly towards the bursa copulatrix, thus preventing their scattering inside the egg channel. The coiled morphology of the proximal portion of the capsule gland duct would probably contribute to slow oocyte transit promoting the provision and arrangement of the oocyte envelopes. Likewise, this particular disposition would increase the length and capacity of the duct, thus enabling the storage of a greater number of oocytes.

The distal portion of the capsule gland duct, which emerges from the parenchymal glandular mass presents an internal epithelium folded in crests, a muscular-connective layer and an external partial epithelial covering. The inner epithelium consists of ciliated and secretory cells. The formers are cylindrical or wedge-shaped with basal nuclei. The bottle-shaped secretory cells exhibit a long neck located between the ciliated cells and a voluminous body with an eccentric nucleus and a cytoplasm filled with secretory granules. The muscular-connective layer is composed of muscular fibers interspersed with collagen fibers and connective cells. The secretions of the distal capsule gland duct would correspond to mucopolysaccharids and acidic mucoproteins (Catalán *et al*., 2000). These secretions would either facilitate penile penetration or provide a suitable vehicle for sperm transfer, whereas in the oviposition process, they would constitute the jelly that binds the eggs in a compact cluster.

According to Buckland-Nicks and Chia (1990), egg encapsulation has evolved together with internal fertilization, in order to ensure reproductive success. The albumen gland-capsule gland complex of *Pomacea canaliculata* is involved in the formation of the layers that surround the fertilized oocytes: both the albumen layer and the egg capsule.

The provision of different oocyte envelopes, which is made up of compounds of different nature, may be correlated with the morphological specialization of the parenchymal secretory cells, and with the ducts of the albumen gland-capsule gland complex. The fine structure of the albumen secretory parenchymal cells and the secretory cells of the albumen gland duct suggest their involvement in the synthesis of the albumen layer, which is made up of a complex mucoprotein material supplemented by significant amounts of calcium. From the same layer Garín *et al*. (1996) have isolated two lipoprotein fractions and another containing lipids and pigments. In this concern, Cheesman (1958) has previously reported in the egg albumen of *Pomacea canaliculata* the presence of a protein-carotenoid complex called ovorubin that would protect the embryo from heat and dehydration, thus preventing the denaturing of proteins. Ovorubin is probably identical to PV-1 of Heras and Pollero (this issue of Biocell). Results obtained in the egg albumen of *Pomacea paludosa* (Meenakshi and Watabe, 1977) also revealed the presence of calcareous spherules made of vaterite.

The albumen layer is the main source of nutrients for the developing embryo since in these freshwater snails the egg yolk has little importance as an energy source. This layer would also provide part of the calcium required for the formation of the protoconch (Tompa, 1980; Turner and McCabe, 1990).

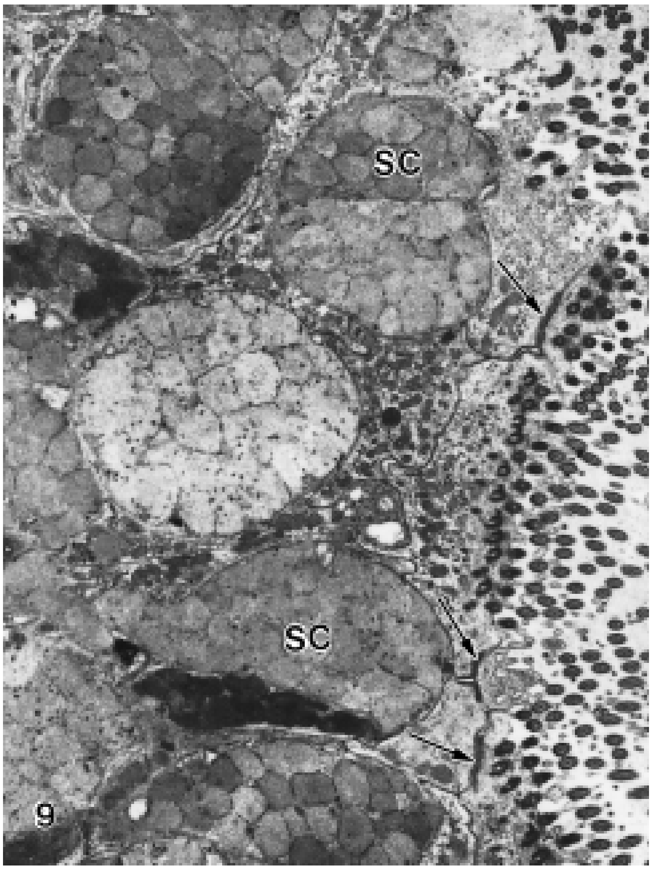
The morphological and histochemical characteristics of the capsule gland duct would demonstrate its participation in the synthesis of the egg capsule, which

FIGURE 9. Coiled intraparenchymal segment of the capsule gland duct. Epithelial layer of one of the halves of the duct. Note the great diversity in the electron-densities of the secretory granules that fill the whole cytoplasm of the secretory cells (SC). Arrows indicate the juxtaluminal junctions. Cilia and microvilli outline the ductal lumen. (X 5,400)

FIGURE 10. Coiled intraparenchymal segment of the capsule gland duct. Epithelial layer of the opposite halve of the duct. Several secretory granules exhibit an electron-dense core (arrowheads). At the apical surfaces the release of the secretory products can be observed (arrows). (X 8,700)

FIGURE 11. Capsule gland duct. Several secretory cells of the epithelial layer exhibit prominent electron-dense deposits of calcium that gradually infiltrate the cytoplasmic matrix, the cellular organelles and the secretory granules. (X 5,400)

FIGURE 12. Capsule gland duct. Ciliated cells showed cilia and microvilli at their luminal surfaces embedded in conspicuous electron-dense calcium deposits. (X 36,500)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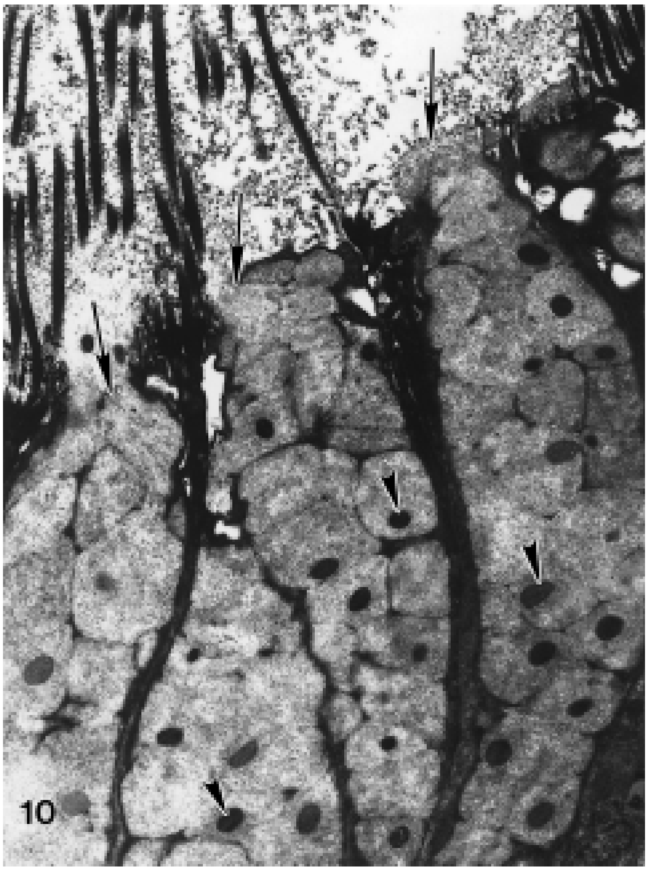
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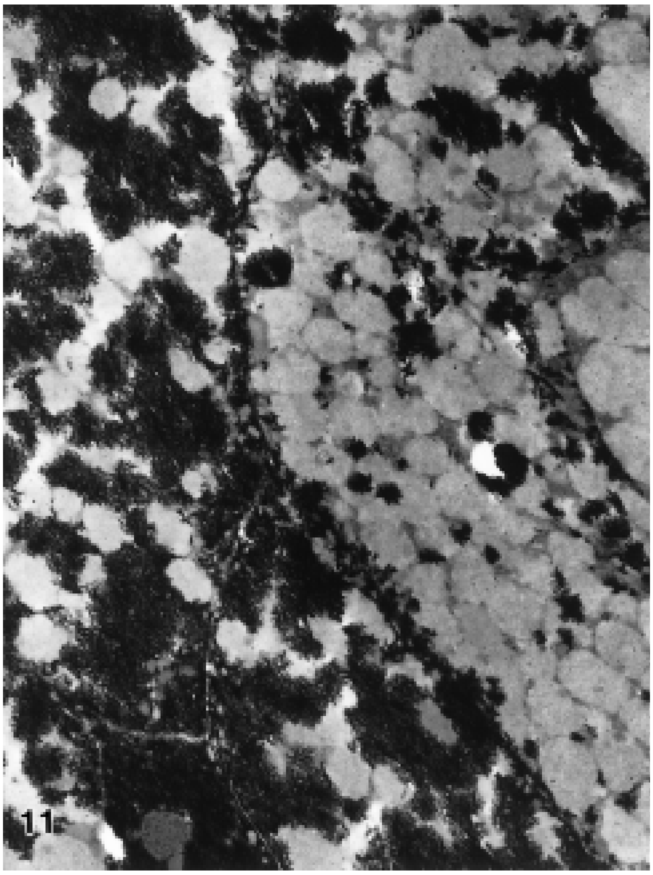
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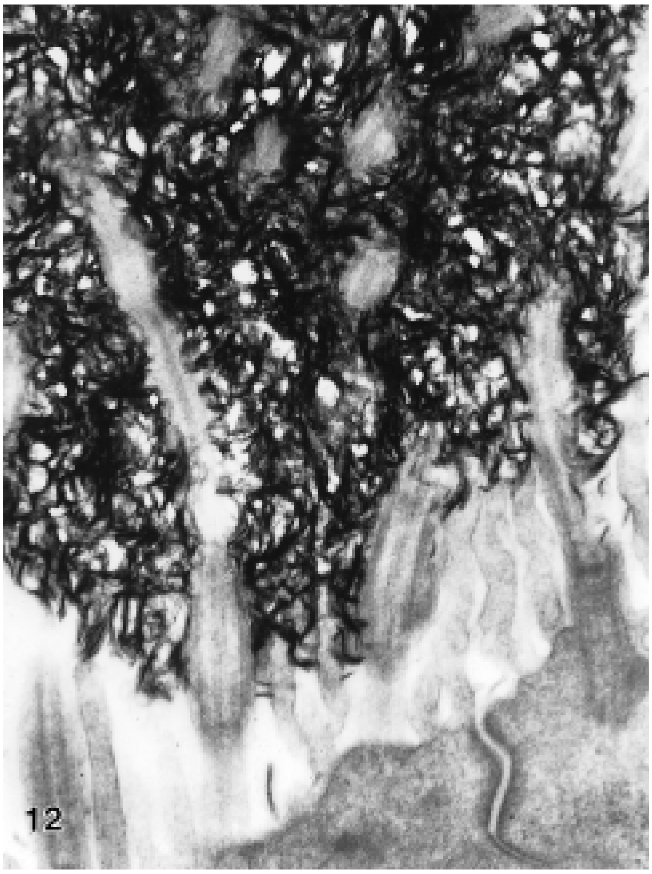
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would be composed of calcium carbonate on an organic matrix of proteins and acid mucopolyssaccharides (Catalán *et al.*, 2001). Meenakshi and Watabe (1977) reported similar findings for the egg capsules of *Pomacea paludosa*.

Likewise, our results are also in agreement with those of Meenakshi and Watabe (1977) who reported that ampullarid snails which lay eggs with calcified egg capsules store calcium in the albumen gland-capsule gland complex to be utilized for egg formation. Although further studies are necessary to establish whether this storage is sufficient to meet all the requirements, or else the snail mobilizes calcium during reproduction from other sources, considering the large amounts of this mineral that should be mobilized for egg formation within a short period of time.

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